Original Article

Comparison of Brilliant Blue G and Trypan Blue during Vitrectomy for Macular Hole Surgery

Chaudhary Nasir Ahmad, Muhammad Shaheer

Pak J Ophthalmol 2014, Vol. 30 No. 4

See end of article for authors affiliations

Correspondence to:
Chaudhary Nasir Ahmad
College of Ophthalmology and Allied Vision Sciences (COAVS),
King Edward Medical University/Mayo Hospital
Lahore
drnasirch1@gmail.com

Purpose: To compare the ease in internal limiting membrane peeling with the use of trypan blue and brilliant blue G.

Material and Methods: This comparative cross sectional study was conducted at Eye Unit III, over duration of two years 1st March 2012 to 28th February 2014, Institute of ophthalmology, Mayo Hospital, Lahore. 60 patients with stage three and four age related macular hole were included in study. They were divided into two groups of thirty patients each. Group A patients underwent internal limiting membrane peeling with the use of brilliant blue G while group B patients underwent internal limiting membrane peeling with adjunctive trypan blue.

Results: The internal limiting membrane peeling was done in 27 of 30 patients in first bite in brilliant blue G group. While only 9 patients underwent successful internal limiting membrane peeling in first bite in the trypan blue group. The internal limiting membrane peeling was done in less than three minutes in 26 patients in brilliant blue G group as compared to 12 in trypan blue group. Collateral damage occurred in 4 patients in trypan blue group as compared to 1 patients only in brilliant blue Group.

Conclusion: Brilliant blue G is the a more useful dye than trypan blue in internal limiting membrane peeling in terms of staining, ease of peel and less side effects.

Key Words: Brilliant blue G, Macular Hole, Vitrectomy,

The human retina and vitreous are bound together by an intervening tissue called internal limiting membrane which acts as a junction for the proliferation of various cells. Diseases of the macula i.e. epimacular membranes and macular holes commonly involve the internal limiting membrane. The constituents of internal limiting membrane include collagen, proteoglycans, basement membrane and plasma membranes of muller cells and myofibrocytes. It is believed that Contraction of these myofibrocytes leads to an enlargement of macular hole thus preventing its closure. Therefore removal of internal limiting membrane from the macula leads to closure of macular holes by inducing gliosis. As the internal limiting membrane is a transparent structure so its removal is a very delicate and difficult procedure as it may lead to inadvertent trauma to retina. Difficult visualization of the internal limiting membrane and its firm attachment to the underlying retina can present technical challenges while trying to peel this membrane.[1]

The problem of visibility of internal limiting membrane has been greatly reduced with the introduction of vital dyes to stain the internal limiting membrane.

Trypan blue is one of the first dyes used to stain the internal limiting membrane. It is successfully being used to stain the anterior lens capsule in surgery of cataracts with absent red reflex.[2] Now a day's trypan

blue is widely being used in posterior segment surgeries as epiretinal or internal limiting membrane peel and cystoids macular oedema surgery. Up till now Trypan blue has not been shown to be associated with any per operative complications such as staining of retinal pigment epithelium cells leading to cell death as with indocyanin green. Previously indocyanin green was being used in epiretinal membrane and macular hole surgery. It was superior to trypan blue in staining the internal limiting membrane but was toxic to retinal pigment epithelium where trypan blue is superior in having no such adverse effects and better visual and functional outcomes.[3]

Brilliant blue G also known as coomasian blue has recently been reported as a tool in chromovitrectomy. It has been widely used for protein staining in biological fields as it non-specifically binds to most proteins. Brilliant blue G stains internal limiting membrane more effectively than other dyes used for staining ILM, ERM and lens capsule. It is easier to handle and is in granular form so that it is easily dissolved at a stable Ph. Histological analysis shows that it has no toxic changes on retinal layers and also successful peeling of ILM occurs without any remnant retinal cells.[4]

Brilliant blue G is comparable to other dyes with regard to visual and functional outcomes but is superior to then as it only and selectively stains ILM. The purpose of this study is to compare ILM peel assisted with trypan blue and brilliant blue G.

MATERIAL AND METHODS

This study was conducted at Mayo Hospital, Institute Of Ophthalmology, Eye Unit III, over duration of two years (1-3-12 to 28-2-14). 60 patients with stage 3 and 4 macular hole were included which were divided into two groups each containing equal number of patients. All patients with idiopathic macular holes were included while patients with rehgmatogenous retinal detachment and myopic / traumatic macular hole and those with ERM were excluded on slit lamp examination of the retina and OCT. Informed consent was taken from the patients followed by detailed history and complete examination of the anterior and posterior segments with the help of slit lamp bimicroscopy and indirect ophthalmoscope. Pre operative OCT was done to stage the macular hole. Post operative OCT was done at 1 week and 1 month to check for anatomical closure. Pre and post operative visual acuity was recorded to check for visual outcome. In the group A patients internal limiting membrane peeling was done with the assistance of brilliant blue G while group B patients underwent internal limiting membrane peeling with adjunctive trypan blue.

In group A patients, after induction of posterior vitreous detachment, air was injected, brilliant blue G (0.5 ml, 0.25 mg/ml) was sprayed over the macular area followed by an air fluid exchange and internal limiting membrane peeling after about one minute of spraying. Similarly the group B patients underwent the same procedure but with the assistance of trypan blue (0.5 ml, 0.06%). In all the patients SF_6 was used after internal limiting membrane peeling and patients were advised face down posture for one week.

RESULTS

60 patients with ages between 40 to 60 years were included in study over a period of one year. In group A there were 18 male and 12 female patients while group B comprised of 22 male and 8 female patients. In group A internal limiting membrane peel was successfully done in 27 patients in first bite while 3 patients underwent the procedure in more than two bites. In group B only 9 patients underwent internal limiting membrane peeling successfully in the first bite (p value 0.037) while 21 patients required more than one bite. Internal limiting membrane peeling was completed within three minutes in 26 patients in group A as compared to 12 patients in group B (p value 0.002). There was 1 iatrogenic break and 3 iatrogenic retinal hemorrhages in group B as compared to only 1 iatrogenic hemorrhage in group A.

DISCUSSION

Vitreo retinal surgeons are commonly performing peeling of internal limiting membrane now a days. Various macular disorders such as macular hole, epiretinal membrane tractional macular oedema and vitreomacular traction syndrome are being treated by doing peeling of internal limiting membrane.[5]

Indocyanin green was first introduced in ophthalmology for the study of choroidal circulation. Later on it was used in the posterior segment surgery to stain the transparent internal limiting membrane during macular hole surgery.[6] In macular hole surgery the concentration of indocyanin green injected into the air or fluid filled vitreous cavity ranges from 0.25 to 0.50 mg/ml.[7]

Table 1: Distribution according to gender

Gender	Trypan Blue	Brilliant Blue
Male	22	18
Female	8	12

Table 2: Ease of ILM peel with respect to time

Time	Trypan Blue	Brilliant Blue
Less than 3 months	12 (40%)	26 (85%)
More than 3 months	18 (60%)	4 (15%)

Table 3: Ease of ILM peel with respect to no of bites

Bites of ILM	Trypan Blue	Brilliant Blue
Single Bite	9 (30%)	27 (90%)
More than one bite	21 (70%)	3 (10%)

Table 4: Ease of ILM peel with respect to collateral damage

Collateral Damage	Trypan Blue	Brilliant Blue
Iatrogenic retinal break	1	None
Iatrogenic retinal hemorrhage	3	1

Table 5: Anatomical closure

Duration of Follow-up OCT (Macular Hole Closure)	Trypan Blue	Brilliant Blue
1 Week	23 (80%)	29 (99%)
1 Month	21 (70%)	28 (95%)

Table 5: Anatomical closure

Visual Acuity	Preoperative	Postoperative (Improved)	Postoperative (Static)
Better than 6/18	38	31	7
Worse than 6/18	22	12	10

Application of Indocyanin green changes the light absorption properties of the ILM and increases the stiffness of the membrane. The indocyanin green potentiated light toxicity can be prevented by using a filter that could block the wavelengths beyond 620 nm.[8]

Trypan blue is a vital stain which has been widely used in ocular surgery. In ocular surgery a concentration of 0.06 to 0.15% is used. Internal limiting membrane staining with trypan blue is subtler than with indocyanin green probably because trypan blue only stains a mild epiretinal membrane above the internal limiting membrane rather than itself.[9]

The latest application of trypan blue in chromovitrectomy is in the staining and localization of retinal breaks during vitrectomy for retinal detachment. 0.15% trypan blue is injected transretinally.

Gandorfer et al concluded in their research that trypanblue staining promoted no ultra structural retinal damage but there were fragments of muller cells adherent to retinal side of internal limiting membrane and muller cell end feet were avulsed and ruptured.[10]

Naryanan et al also examined the effect of trypan blue exposure on human RPE cells using the dye exclusion method and concluded that trypan blue at all concentrations did not affect RPE cells with or without light exposure.

Brilliant blue G has emerged as a leading dye among all the vital dyes in staining the internal limiting membrane during vitrectomy. Brilliant blue G shows no retinal toxicity or adverse effects such as ganglion cell death and retinal pigment cell atrophy which is seen with the use of other dyes.

Recently modifications have been made in Brilliant blue G by mixing it with 10% dextrose and heavy water thereby making it dense than vitreous and intraocular fluids. This modification serves two purposes. First the dye accumulates on the posterior pole rather than spreading in the vitreous thus making the macular contact time prolonged. Secondly less amount of dye is used both in terms of volume and concentration.[11] Atul Kumar[12] et al compared brilliant blue G and triamcinolone acetonide in internal limiting membrane peeling. It was concluded that there was a statistically significant difference in the visual acuity of both the groups making brilliant blue g with better visual outcome.

Machaida S[13] et al compared the cone electroretinograms after ICG, BBG and TA assisted macular

hole surgery. The a and b wave potentials were generally decreased in all the patients but the photopic sensitivity response was significantly decreased in patients undergoing surgery with the assistance of ICG as compared to normal responses in patients treated with adjunctive BBG. Baba T[14] et al compared vitrectomy with brilliant blue G and indocyanin green and its effect on functioning of the eye. The best corrected visual acuity was better in the brilliant blue G group. The mean retinal sensitivity significantly improved in the BBG group. They concluded that brilliant blue G was better in making the visibility of internal limiting membrane as well as having minimal side effects. Doaa Awaad[15] et al studied the toxic effects of brilliant blue G and trypan blue. In their study the exposed the cultured human retinal pigment epithelial cells to the trypan blue and brilliant blue g at varying concentrations and time. They concluded in their study that trypan blue was more toxic to the cultured human retinal epithelial cells at all concentrations and times of exposure. Also brilliant blue g was more safe in maintaining the integrity of Muller cells after internal limiting membrane peeling for macular hole.

Shukla R[16] et al compared trypan blue, brilliant blue g and indocyanin green in their ease in internal limiting membrane peeling.

The brilliant blue g group had a better post operative visual acuity and less visual decline as compared to other groups. Based on these observations it was concluded that BBG was comparable with trypan blue in optimizing visual function while it was similar to ICG in ease of internal limiting membrane peeling. But it was associated with less side effects and toxicity as compared to other two groups.

In our study we compared brilliant blue g and trypan blue in internal imiting membrane peeling with respect to staining, timing of membrane eeling, number of bites of internal limiting membrane during peeling and collateral damage (retinal break or hemorrhage).

It was observed that 26 patients underwent membrane peel within three minutes while 4 surgeries took more than five minutes in brilliant blue G group. Similarly 12 patients underwent membrane peel within three minutes and the rest took more than three minutes in the trypan blue group.

27 surgeries were completed with first bite while 3 required more than one bite in the brilliant blue g group. Similarly only 9 membrane peel were done with first bite while the rest 21 required more than one bite. There was 1 iatrogenic retinal break during surgery while 3 patients had iatrogenic retinal hemorrhage in the trypan blue Group as compared to only one iatrogenic retinal hemorrhage in the brilliant blue G group during surgery.

Follow up OCT was done to see the anatomical closure. 29 macular holes in brilliant blue G group and 28 macular holes in the trypan blue group were closed on OCT after one month of surgery.

Post operatively visual acuity improved in 43 patents out of 60 while 17 patients showed no improvement or worsening in visual acuity.

CONCLUSION

Based on above observations in our studies we conclude that brilliant blue G is more efficacious in staining the internal limiting membrane leading to a statistically significant ease in visibility, peeling, shorter surgery time and less side effects and less collateral damage (though not statistically significant) but still significant clinically as compared to trypan blue.

Author's Affiliation

Dr. Chaudhary Nasir Ahmad
College of Ophthalmology and Allied Vision Sciences (COAVS),
King Edward Medical University / Mayo Hospital, Lahore

Dr. Muhammad Shaheer
College of Ophthalmology and Allied Vision Sciences (COAVS),
King Edward Medical University / Mayo Hospital, Lahore

REFERENCES

1. **Kagimoto HTS, Hishatomi T, Eneida H, Ishibashi T.** This potent stain is easy to handle with minimal side effects. Retina Today. 2001; 45-48.
2. **Gerrit RJM, Peter WTD, Jan HP, W Houdijn B.** Trypan blue staining to visualize capsulorhexis during cataract surgery. Journal of cataract and refractive surgery. 1999; 25: 7-9.
3. **Jeffery SG, Alain AP, John RG, Alexandar JM, Cindy MLH.** Comparison of in vitro toxicity of indocyanin green to that of trypan blue in human retinal pigment epithelium cell cultures. American Journal of Ophthalmology. 2004; 138: 64-9.
4. **Nithani P, Vashisht N, Khunduja S, et al.** Brilliant blue G assisted peeling of internal limiting membrane in macular hole surgery. Indian J Ophthalmol. 2011; 59: 158-60.

5. **Abdelkader E, Louis N.** Internal limiting membrane peeling in vitreoretinal surgery. Surv Ophthalmol. 2008; 53: 368-96.
6. **Kadonosono K, Ito N, Uchio E, Nakamura S, Ohno S.** Staining of internal limiting membrane in macular hole surgery. Arch Ophthalmol. 2000; 118; 1116-8.
7. **Ando F, Sasano K, Suzuki F.** Indocyanin green assisted ILM peeling in macular hole surgery revisited. Am J Ophthalmol. 2004; 11: 2246-53.
8. **Wollensak G.** Vital dyes in vitreoretinal surgery. Dev Ophthalmol. 2008; 42: 82-90.
9. **Farah M, Maia M, Furlani B, Bottos J, Meyer C, Lima V, Penha F, Costa E, Rodrigues E.** Current concepts of trypan blue in chromovitrectomy. Dev Ophthalmol. 2008; 42: 91-100.
10. **Gandorfer R, Rohleder M, Charteris DG, Sethi C, Kempik A, Luthert P.** Staining and peeling of the internal limiting membrane in cat eye. Curr Eye Res 2005; 30: 977-88.
11. **Shukla D, Kelliath J, Patwardhan A, Kannan NB, Thayyil SB.** A preliminary study of heavy brilliant blue G for internal limiting membrane staining in vitreoretinal surgery. Indian Jr of Ophthalmology. 2012; 60: 531-4.
12. **Kumar A, Gogia V, Shah VM, Sinha S.** Brilliant blue G versus triamcinolone assisted ILM peeling: A comparitive evaluation in macular hole surgery. World journal of retina and vitreous. 2011; 1: 1-4.
13. **Machida S, Toba Y, Nishimura T, Ohzeki T, Murai K, Kurosaka D.** Comparisons of cone electrograms after indocyanin green, Brilliant blue G, or triamcinolone assisted macular hole surgery. Greafe arch clin exp Ophthalmol. 2014; 252: 1423-33.
14. **Baba T, Hagiwara A, Sato E, Arai M, Oshitari T, Yamamoto S.** Comparison of vitrectomy with indocyanin green or brilliant blue g on retinal microvasculature and function of eyes in macular pathology. Ophthalmology. 2012; 119: 2609-15.
15. **Awaad D, Schrader I, Bartok M, Mohr A, Gabel D.** Comparitive toxicology of brilliant blue , trypan blue and their combination together with polyethylene glycol on human pigment epithelial cells. Investigative science and visual ophthalmology. 2011; 52: 4085-90.
16. **Shukla D, Kelliath J, Neelakantan N, Naresh K, Ramasamey K.** A comparison of brilliant blue g, trypan blue and indocyanin green dyes to assist internal limiting membrane peeling during macular hole surgery. Retins. 2011; 31: 2021-5.